

Computational study of small molecule binding for both tethered and free conditions

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Using a calix[4]arene-benzene complex as a test system we compare the potential of mean force for when the calix[4]arene is tethered versus free. When the complex is in vacuum our results show that the difference between tethered and free is primarily due to the entropic contribution to the potential of mean force resulting in a binding free energy difference of 6.5 kJ/mol. By contrast, when the complex is in water our results suggest that the difference between tethered and free is due to the enthalpic contribution resulting in a binding free energy difference of 1.6 kJ/mol. This study elucidates the roles of entropy and enthalpy for this small molecule system and emphasizes the point that tethering the receptor has the potential to dramatically impact the binding properties. These findings should be taken into consideration when using calixarene molecules in nanosensor design.

I. INTRODUCTION

Calixarenes are macrocycles that are of interest due to the fact that they can be easily synthesized and can be functionalized to selectively bind neutral or ionic analytes; see review refs 1,2,3,4,5. One use for calixarenes that is of specific interest to the current study is in nanosensor design (e.g., refs 6,7,8,9). Calixarenes are typically used in nanosensors by decorating the nanomaterial with gold and then tethering the calixarenes to the gold surface. A reasonable question that is also the motivation for our study is: How does tethering calixarene to a surface affect the binding properties of the calixarene to analytes? This is an important question considering that typically a researcher may only have knowledge of binding properties for free (i.e., not tethered) conditions.

For the current study we compare the effects of tethering a calix[4]arene on the binding properties for both in vacuum and in water. Note that the “[4]” means that there are four aromatic rings in the structure. To our knowledge there are no previous studies that have determined the affects of such tethering on the binding properties of calixarenes. Thus, we computed the potential of mean force (PMF) for calix[4]arene-benzene binding for four cases: (i) in vacuum with the calix[4]arene tethered; (ii) in vacuum with the calix[4]arene free (i.e., not tethered); (iii) in water with the calix[4]arene tethered; and (iv) in water with the calix[4]arene free. Our results below show that when the complex is in vacuum the difference between tethered and free is due primarily to the entropic contribution to the potential of mean force resulting in a binding free energy difference of 6.5 kJ/mol. By contrast, when the complex is in water our results suggest that the difference between tethered and free is due entirely to the enthalpic contribution resulting in a binding free energy difference of 1.6 kJ/mol.

II. COMPUTATIONAL METHODS

The initial structure for the calix[4]arene-benzene complex was obtained from experimental X-ray crystallography (personal communication from Pam Shapiro’s lab at University of Idaho). The necessary simulation topologies for both the calix[4]arene and benzene were then generated by the PRODRG server¹⁰. We then modified the partial charges to be consistent with the GROMOS-96 43A1 forcefield¹¹, e.g., all CH₃ groups were set to zero partial charge. The GROMACS simulation package version 3.3.3¹² was used for all molecular dynamics simulations described below with the default GROMOS-96 43A1 forcefield¹¹.

For the vacuum simulations the calix[4]arene-benzene complex was first minimized using steepest decent for 1000 steps. For subsequent production simulations all Van der Waals and electrostatic interactions were computed, i.e., no cutoffs were used. A timestep of 1.0 fs was utilized with no constraints. The temperature was maintained at a constant value using Langevin dynamics¹³ with a friction coefficient of 1.0 amu/ps.

For the simulations in water the calix[4]arene-benzene complex was solvated in a cubic box of SPC water¹⁴ of approximate initial size 4.5 nm a side. The system was then minimized using steepest decent for 1000 steps. To allow for some equilibration of the water the system was then simulated for 100 ps with the positions of all heavy atoms in the complex harmonically restrained with a force constant of 1000 kJ/mol/nm². For this equilibration simulation the pressure was maintained at 1.0 atm using the Berendsen algorithm¹⁵. Subsequent production simulations were carried out with the volume fixed at the final value from the equilibration. For all water simulations the LINCS algorithm¹⁶ was used to constrain hydrogens to their ideal lengths allowing the use of a 2.0 fs timestep. The temperature was maintained at a constant value using Langevin dynamics¹³ with a friction coefficient of 1.0 amu/ps. Particle mesh Ewald¹⁷ was used for electrostatics with a real-space cutoff of 1.0 nm and a Fourier spacing of 0.1 nm. Van der Waals interactions used a cutoff with a smoothing function such that the interac-

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tions smoothly decayed to zero between 0.75 nm and 0.9 nm. Dispersion corrections for the energy and pressure were utilized¹⁸.

To perform the tethered simulations for both vacuum and in water we harmonically restrained the two sulfur atoms shown in 1 using a force constant of 10,000 kJ/mol/nm². The purpose is to mimic the effect of the calix[4]arene binding to a gold surface. This harmonic restraint on the sulfur atoms was not present for the free simulations.

A. Generating PMF estimates

We computed all PMFs using umbrella sampling and weighted histogram analysis (WHAM)²⁰. Our technique for estimating the PMF using WHAM is described in ref 21. Briefly, the GROMACS 3.3.3 software package¹² was modified to provide a harmonic biasing potential $U_r(r) = 0.5k_r(r - r_0)^2$ which depends only on the center of mass separation r between the calix[4]arene and the benzene. For all PMF estimates we used a total of 33 windows $r_0 = 0.40, 0.45, 0.50, \dots, 1.95, 2.00$. For the vacuum system each window was simulated for 32.0 ns; 16.0 ns were discarded for equilibration and 16.0 ns were used for the WHAM analysis. For the water system each window was simulated for 4.0 ns; 2.0 ns were discarded for equilibration and 2.0 ns were used for the analysis. For all PMF estimates below the biasing potential U_r used a force constant $k_r = 3000$ kJ/mol/nm² and the estimates include the $2\ln(r)$ Jacobian correction^{21,22}.

Note that for the simulations of the complex in water the system size prevents the long simulation times necessary to obtain converged PMFs without additional restraints. Thus, for the water simulations (but not for the vacuum simulations) we utilized an axial restraint that keeps the benzene on the binding axis relative to the calix[4]arene as described in ref 21. Use of this restraint means that it is not valid to directly compare the vacuum and water PMFs. However, it is still valid to compare the tethered and free conditions for water which is the purpose of this study.

B. Estimating entropic and enthalpic contributions

To estimate the entropic contribution to the PMF $T\Delta S(r)$ we used the fact that the entropy is related to the derivative of the PMF $\Delta G(r)$ with respect to system temperature T (see also refs 23,24),

$$T\Delta S(r) = -T \left(\frac{\partial \Delta G(r, T)}{\partial T} \right). \quad (1)$$

This derivative was numerically estimated by computing the PMF at three temperatures 270 K, 300 K and 330 K and then using a three-point finite difference approximation. The enthalpic contribution $\Delta H(r)$ was then

estimated via

$$\Delta H(r) = \Delta G(r) + T\Delta S(r). \quad (2)$$

C. Uncertainty estimation

The uncertainty for ΔG , $T\Delta S$ and ΔH were estimated by computing the standard error over independent trials. For both the tethered and free conditions in vacuum 10 independent estimates of the PMF were generated at each of the three temperatures (i.e., 30 PMF estimates tethered and 30 free). For both the tethered and free conditions in water five independent estimates of the PMF were generated at each of the three temperatures (i.e., 15 PMF estimates tethered and 15 free).

III. RESULTS AND DISCUSSION

Results for both vacuum and water simulations are shown in 2. The binding free energy differences between tethered and free conditions $\Delta\Delta G_{\text{bind}}$ for vacuum and water were obtained by numerically integrating the $\Delta G(r)$ curves from $r = 0.4$ to $r = 1.9$ nm. The entropic and enthalpic contributions to the PMF were computed using eqs (1) and (2).

Results for the calix[4]arene-benzene complex in vacuum are shown in 2a and reveal two major differences between the free and tethered conditions. First, when the PMF plateaus ($r > 1.5$ nm) both the entropic and enthalpic contributions for the free conditions is larger than for tethered. Note that the free entropic contribution is larger than tethered by approximately 15 kJ/mol, and the enthalpic contribution is larger by about 8 kJ/mol. Thus, it is primarily the entropic difference between free and tethered that leads to the more favorable binding for tethered conditions. The second difference between free and tethered conditions occurs at $r \approx 0.6$ nm where the entropic and enthalpic contributions to the PMF increase dramatically for free as compared to tethered conditions. This difference can be understood by noting that the benzene is at the (wider) outer edge of the binding pocket at $r \approx 0.6$ nm; see 1. Benzene is just outside the binding pocket at $r \approx 0.7$ nm where entropic and enthalpic contributions for the free condition have a narrow flat region. We believe that this difference between the free and tethered can be attributed to the fact that the tethering of the calix[4]arene provides a more rigid binding pocket than under free conditions leading to a sharp increase in entropy under free conditions as the benzene reaches the outer edge of the pocket.

Our vacuum results suggest that the free energy of binding under tethered conditions is more favorable than free by $\Delta\Delta G_{\text{bind}} = -6.5$ kJ/mol, due primarily to the entropic contribution to the PMF. Thus, if one wishes to design a gas phase nanosensor using calix[4]arenes we strongly suggest testing the binding properties under tethered conditions.

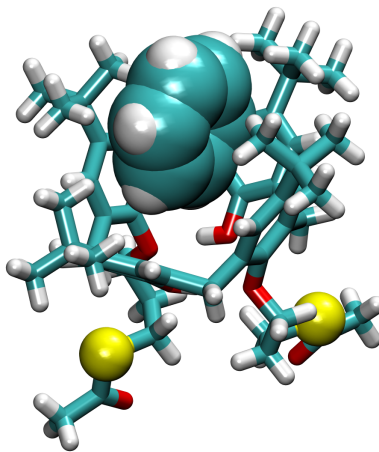


FIG. 1: The calix[4]arene-benzene model system used for the current study. The calix[4]arene molecule has a basket shaped binding pocket and can be functionalized to bind both neutral and ionic analytes. The two sulfur atoms are shown in a larger size and allow the calix[4]arene to be tethered to a gold surface. The difference between the tethered and free simulations in the current study is that these sulfur atoms were harmonically restrained to the position shown in the figure during the tethered simulations but were not restrained for the free simulations. This image was generated using VMD¹⁹.

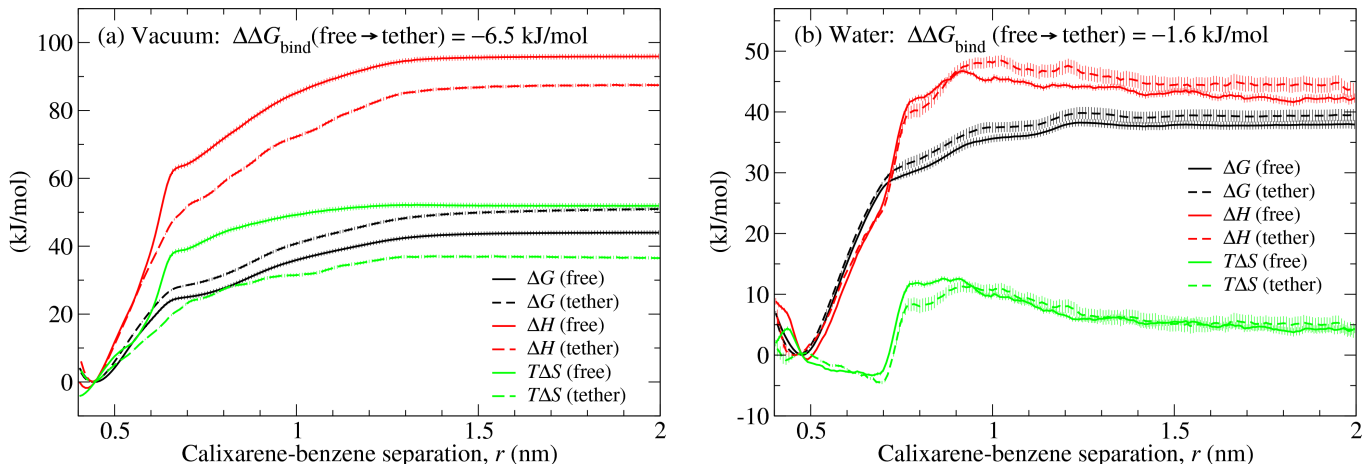


FIG. 2: The calix[4]arene-benzene potential of mean force (black) showing the enthalpic (red) and entropic (green) contributions. Both tethered (dashed line) and free (solid line) conditions are shown. The error bars are the standard error obtained from performing multiple independent simulations. (a) Simulation results in vacuum. Due primarily to the entropic contribution there is a free energy difference of 6.5 kJ/mol between tethered and free conditions. (b) Simulation results in water. Due primarily to the enthalpic contribution there is a free energy difference of 1.6 kJ/mol between tethered and free conditions.

Results for the calix[4]arene-benzene complex in water are shown in 2b. The only appreciable difference between tethered and free conditions is the enthalpic contribution when the PMF plateaus ($r > 1.5$ nm). Interestingly, there is no difference (within error) between the free and tethered entropic contributions to the PMF for $r > 1.5$ nm. Apparently the entropy of the water molecules completely counters the entropy-dominant effects seen in the vacuum system. We believe this is attributed to the fact that the effective volume available to the water molecules is reduced when the benzene is completely dissociated from the calix[4]arene; this also leads to the entropy de-

crease observed in the PMFs for both tethered and free between $r \approx 0.9$ and $r \approx 1.5$ nm.

Our results in water suggest that the free energy of binding under tethered conditions is more favorable than free by $\Delta\Delta G_{\text{bind}} = -1.6$ kJ/mol. In contrast to vacuum this free energy difference is due entirely to the enthalpic contribution to the PMF.

Note that we do not expect that our results are completely general and thus different receptor-compound complexes will likely lead to differences from our results above. However, this does not change our general conclusion that one must test binding properties under the

desired conditions.

IV. CONCLUSION

We have studied the effects of tethering on small molecule binding properties using a calix[4]arene-benzene complex as a test system. Simulations of the complex in vacuum and in water were performed and the potential of mean force (PMF) curves were computed and compared for tethered and free conditions.

Our results for the calix[4]arene-benzene complex in vacuum show that the primary difference between free and tethered conditions is the entropic contribution to the PMF. Thus, in vacuum the free energy of binding under tethered conditions is more favorable than free by $\Delta\Delta G_{\text{bind}} = -6.5$ kJ/mol. By contrast, when the calix[4]arene complex is in water the only substantial difference between free and tethered conditions is the enthalpic contribution to the PMF. Thus, in water the free energy of binding under tethered conditions is more favorable than free by $\Delta\Delta G_{\text{bind}} = -1.6$ kJ/mol.

This study elucidates the roles of entropy and enthalpy

under tethered and free conditions for both vacuum and in water. Our results show substantial differences in binding properties between tethered and free conditions, especially in vacuum. Thus, if one wishes to design a gas phase or aqueous nanosensor using calix[4]arenes we suggest that the binding properties of the calix[4]arene should be tested under tethered conditions.

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